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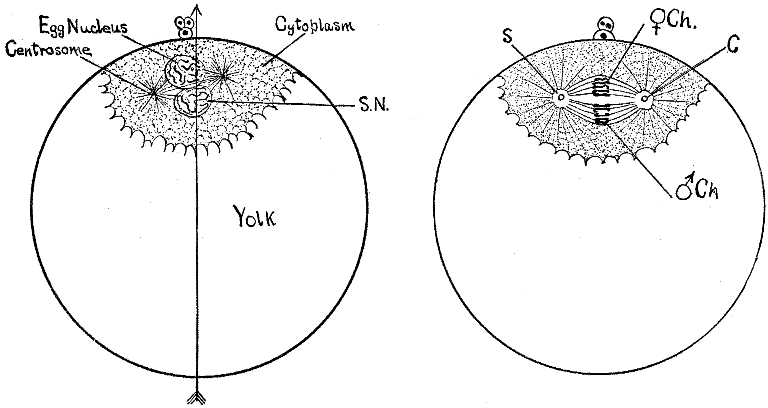
THE INDIVIDUALITY OF THE GERM NUCLEI DURING THE CLEAVAGE OF THE EGG OF CREPIDULA.

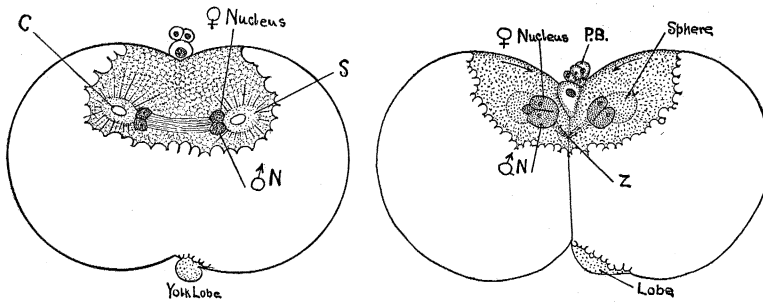
EDWIN G. CONKLIN.

HAECKER ('**92**) and Rückert ('**95**) have made known the interesting fact that the germ nuclei of Cyclops do not fuse but preserve their individuality throughout a considerable portion of the cleavage of the egg. Herla ('**93**) and Zoja ('**95**) have shown that the paternal and maternal chromosomes of Ascaris remain distinct at least as far as the 12-cell stage. These observations are of the greatest significance and, so far as they go, establish Boveri's hypothesis ('**91**), "that in all cells derived in the regular course of division from the fertilized egg, one-half of the chromosomes are of strictly paternal origin, the other half of maternal." So far as I am aware, similar observations have not hitherto been made in the case of other animals.[1]

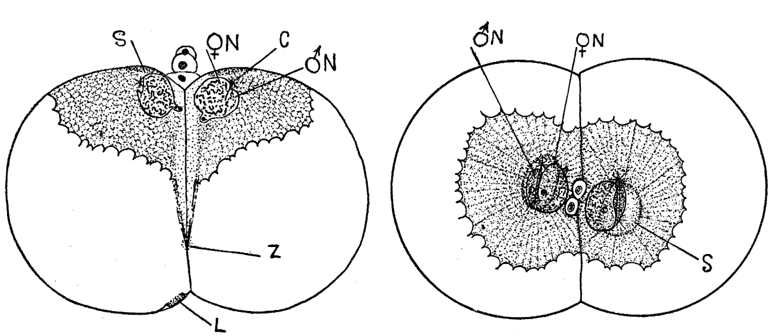
[1] Rückert calls attention to the fact that partially cleft nuclei are found in the figures of various authors, particularly those of Fol ('**79**) on Toxopneustes, of Bellonci ('**84**), and of Kölliker ('**89**) on Siredon. Of course no one of these observers has interpreted these figures as showing the independence of the germ nuclei, and some of the figures referred to by Rückert probably do not show this phenomenon. For example, only one of Fol's figures (Pl. VII, Fig. 7) shows a dual nucleus, while the figure in Kölliker's text-book (Fig. 36) is probably a case of the indentation of the nuclear membrane opposite the centrosomes in the early prophase, a thing which frequently happens. Bellonci's Figs. 1 and 20 show an indentation on one side of the nucleus which may correspond to a division between the germ halves, though this must be regarded as more or less doubtful.



FIGS. 1 and 2. — Prophase and Metaphase of First Cleavage. *S.N.* = Sperm Nucleus. *Ch.* = Chromosomes. *S.* = Sphere. *C.* = Centrosome.

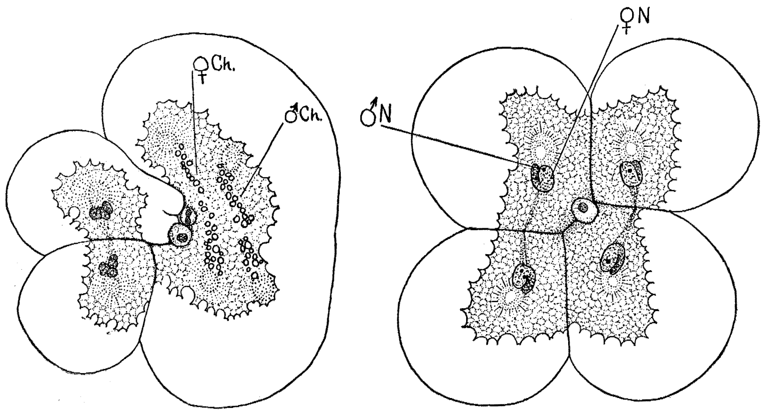


FIGS. 3 and 4. — Anaphase and Telophase of First Cleavage, showing dual nuclei, centrosomes (*C*) and spheres (*S*), "Zwischenkörper" (*Z*), bending of spindle axis, and progressive absorption of yolk lobe.

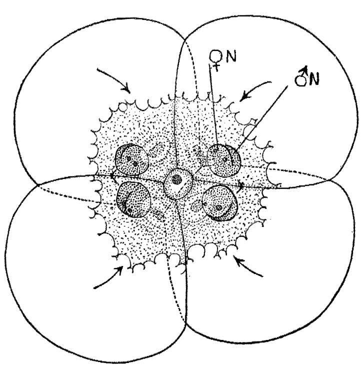


FIGS. 5 and 6. — Prophase of Second Cleavage, showing dual nuclei with central spindle lying in groove between the halves; Fig. 5 viewed from one side, Fig. 6 from animal pole. The spheres (*S*) lie over the nuclei and immediately under the cell wall; the spindle axis is bent on itself, and the "Zwischenkörper" (*Z*) is carried nearly to the vegetal pole; the nuclei shows processes projecting toward the "Zwischenkörper," and the yolk lobe (*L*) is almost completely absorbed.

In Crepidula plana I have observed a separateness of the germ nuclei in certain stages of the nuclear cycle which is of such a character that it may lead to the discovery of similar phenomena in other animals. This separateness is most easily



FIGS. 7 and 8. — Anaphase and Telophase of Second Cleavage. Fig. 7, an abnormal egg in which the left half has divided normally and the chromosomal vesicles of the daughter-nuclei are fusing; in the right half the division figure is double with four spheres and two groups of chromosomal vesicles which have not fused; there are thirty chromosomal vesicles in each group. Fig. 8, a normal egg showing the egg and sperm constituents in each of the daughter-nuclei.



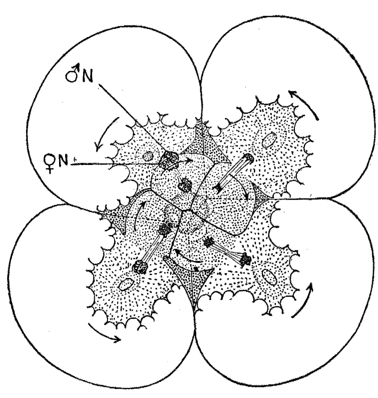


FIG. 9. — Telophase of Second Cleavage; centrosomes, spheres, and nuclei rotating in direction of arrows.

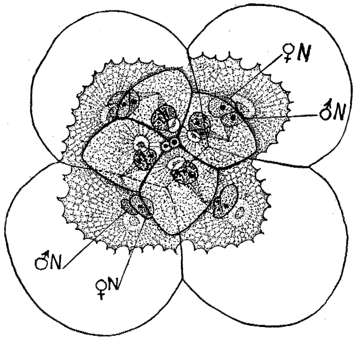
FIG. 10. — Anaphase of Third Cleavage. Egg and sperm constituents of nuclei indicated.

observed in the telophase of each division, though in some cleavage cells it may be seen in the prophase also, or even throughout the resting period. At the time when the daughter-nuclei are being formed the chromosomal vesicles fuse into two

groups which are closely pressed together but are still separated by a partition wall (Figs. 4, 8, *et seq.*), as Rückert has shown to be the case in Cyclops. Gradually this partition wall disappears, being preserved longest on that side of the nucleus nearest the centrosome (Fig. 5). Here a groove is formed on one side of the nucleus which marks the line of contact between the two halves. In some cleavage cells this groove is visible throughout most of the resting period (Figs. 4, 9); in others it disappears during the greater part of the resting period, though it may reappear in the following prophase (Figs. 5, 6); in all cases, however, the partition wall and groove reappear in the next succeeding telophase, when it is formed again in the manner described above. I have observed the double character of the nucleus in the telophase of every cleavage up to the 29-cell stage (Figs. 1–16), and in several of the later cleavages up to the 60-cell stage, and I have no doubt that it is found in all the later cleavages, though it becomes more difficult to see as the nuclei grow smaller. While the halves of these double nuclei occupy similar positions relative to each other at corresponding stages in any cell generation, they occupy different positions at different stages and in different generations; consequently the position of the groove or partition wall which separates the halves of the double nuclei can be satisfactorily studied only in preparations of entire eggs, which may be observed from all sides. All the figures which illustrate this paper are therefore of entire eggs, though many isolated cases of double nuclei have been observed and studied in actual sections.

On each side of the partition wall which divides these double nuclei there is usually a single small nucleolus; these two nucleoli persist long after the disappearance of the partition and frequently throughout the whole of the resting period. In most if not all of the early cleavages there are two, and only two, nucleoli present in the telophase (Figs. 3, 4); but if this is succeeded by a very long resting period the number may increase to more than two, or all may fuse into a single enormously large one.

It still remains to show that these double nuclei really



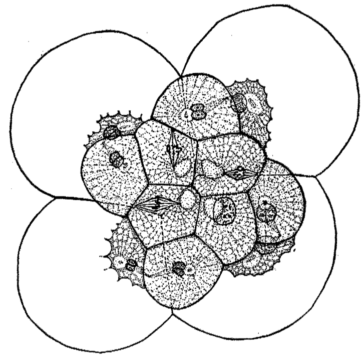
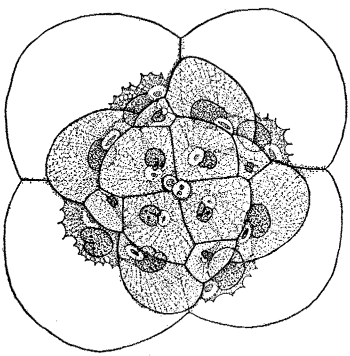


FIG. 11. — Telophase of Third Cleavage. Egg and sperm constituents of nuclei indicated; also bending of spindle axis and rotation of centrosomes and nuclei.

FIG. 12. — Telophase of Fourth Cleavage and Prophase of division of First Quartette cells. The nuclei in the telophase are dual, though from this stage on the egg and sperm constituents cannot be identified with certainty.



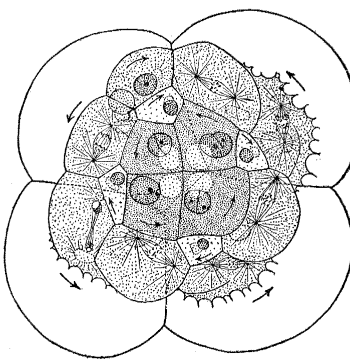
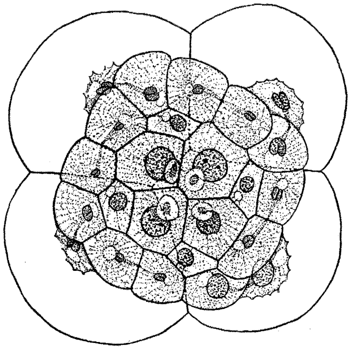


FIG. 13. — Telophase of Division of First Quartette. Dual nuclei in each daughter-cell.

FIG. 14. — Subdivision of Second Quartette and formation of Third. Dual character of nuclei shown in apical cells.



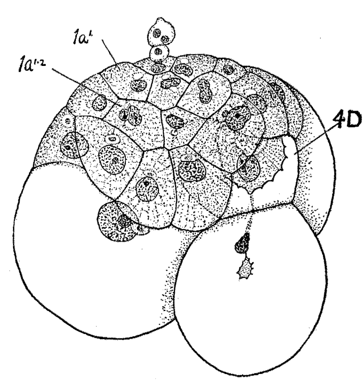


FIG. 15. — Telophase of Division of Second Quartette and of formation of Third; dual nuclei shown in almost all of these cells.

Fig. 16. — Telophase of formation of the Mesentoblast Cell (4 D) and of the second division of the First Quartette ($1\,a^1$, $1\,a^{1\cdot2}$, etc.); dual nuclei shown in both cases.

represent the egg and sperm nuclei which have not yet lost their individuality. This cannot be demonstrated in Crepidula, for the reason that this double character is not apparent at every stage in the nuclear cycle, but it is extremely probable, as the following observations will show:

1. In the first cleavage the germ nuclei do not fuse but remain distinct throughout the prophase, and even in the metaphase they are represented by separate groups of chromosomes (Figs. 1, 2); in the early anaphase these groups of chromosomes can no longer be distinguished, though I think they must still remain separate, for the nuclei are clearly double in the immediately following late anaphase and telophase (Figs. 3, 4). The position of the partition wall in these double nuclei corresponds to the plane of contact between the germ nuclei; the egg nucleus always lies more or less above the sperm nucleus, and in the telophase of the first cleavage one-half of each double nucleus overlaps the other half to a greater or less extent (Figs. 1–4). It is probable that the upper half represents the egg nucleus, and the lower half the sperm nucleus, and in all the later cleavages it is probable that the half of the nucleus which lies nearest the animal pole is from the egg, and the other half from the sperm.

2. The groove which is found on one side of the nucleus in the telophase of the first cleavage (Fig. 4) persists well into the resting stage, and a corresponding groove is found in the same position in the prophase of the second cleavage (Figs. 5, 6). The central spindle for the second cleavage lies in this groove (Fig. 6), and thus the amphiaster actually lies in the only plane in which it would be possible to halve the two parts of the double nuclei. This very fact shows that each half of a double nucleus is represented in the daughter-nuclei, and it strongly suggests that the two parts of the daughter-nuclei are derived directly from the corresponding parts of the mother-nucleus (*cf.* Figs. 6, 8). The fact that the central spindle lies in the groove separating the halves of the nucleus has been observed in the first, second, third, and fourth cleavages, and is undoubtedly a general phenomenon. There is no reasonable ground for doubting that the two parts of every double nucleus are

derived from corresponding parts of a mother-nucleus, and so on back to the egg and sperm nuclei in the first cleavage.

Since the descendants of the germ nuclei are halved at every division, it follows that successive divisions of the double nuclei cannot be at right angles to one another, since this would lead to an unequal division of the halves, or even to a division along the plane of contact between the halves. Such an unequal division might be prevented in cleavages which successively alternate in direction by the rotation of the nucleus during the resting period, or by the rotation of the spindle in the early stages of mitosis.[1] As a matter of fact both of these methods occur in Crepidula. The nucleus usually rotates during the rest through 90°, so that although successive nuclear spindles are at right angles to one another the axis of every spindle lies in the same nuclear axis (*cf.* Figs. 3, 4, 8, 9); but in some cases the nuclear spindle does not lie in its definitive position when first formed but undergoes extensive rotation after its formation. While it is not susceptible of absolute proof, since the partition wall is absent during the later stages of the rest, it is highly probable that the plane in which all nuclear spindles lie is the plane of contact between the two halves of every nucleus.

3. In certain abnormal cleavages the double nuclei are really two entirely separate nuclei lying side by side within a single cell. Such binucleated cells may occasionally be found with the nuclei in the height of the rest, though they are more usual in the telophase or early resting period. There is usually but a single sphere and centrosome in such cells, though in one case of pathological mitosis which I have seen (Fig. 7) there are two mitotic figures side by side; the chromosomes which have reached the stage of the chromosomal vesicles have not aggregated at the poles of these spindles, but are scattered along their whole length. There are thirty of these

[1] Rückert finds that the nuclei rotate in Cyclops even after the spheres have reached their definitive positions at the poles of the spindle; I have never observed in Crepidula a rotation of the nuclei, independent of the spindles, at so late a stage in the cell cycle.

chromosomes in each spindle, the same number that is found in each germ nucleus.

4. In each of the germ nuclei, before they come into contact, there is a single nucleolus ; these nucleoli disappear in the prophase of the first cleavage, but in the succeeding telophase a single nucleolus generally appears in each half of each daughter-nucleus. The same is true of the succeeding cleavages, so that each nucleus throughout the cleavage usually has two nucleoli in the telophase or early resting stage, though the number may vary in the later resting period, as pointed out above. The fact that there is a single nucleolus in each germ nucleus, and that there is usually a single nucleolus in each half of the double nuclei of the cleavage, may possibly indicate that these halves are each derived from one of the germ nuclei. Since the nucleoli as such do not persist throughout the mitosis, may it not be possible that there is some achromatic structure in connection with them which does persist and form the basis for the new nucleoli which appear in the daughter-nuclei?

These facts make it very probable that the germ nuclei of Crepidula preserve their individuality throughout the cleavage, though their separateness may be apparent only or chiefly in a single stage of the nuclear cycle, *viz.*, the telophase. Further, it is possible, even in an advanced stage of the cleavage, to determine with considerable probability which part of a double nucleus is derived from the egg and which from the sperm, the egg half always lying nearer the animal pole than the sperm half. Finally the initial position of the mitotic spindle seems to be determined by the relative positions of the halves of the double nuclei, since the spindles when they first appear lie in the plane of contact between the two halves ; the final position of the spindle and the direction of division are determined by the movements of the cytoplasm.

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